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RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2014

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Abstract:

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to primarily assess the identification of the fish viruses: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis virus (EHNV), spring viraemia of carp virus (SVCV), and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was structured with the aim of assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV) and Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by biomolecular methods (PCR based). 41 laboratories participated in PT1 while 40 participated in PT2.
The tests were sent from the EURL in the beginning of October 2014.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively, see table 1. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the listed fish viruses VHSV, IHNV and ENHV (Council Directive 2006/88/EC) and the non-listed viruses SVCV and IPNV if present in the ampoules, bearing in mind that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision 2001/183/EC using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.
If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2014. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et al. (2004) for VHSV and in Kurath et al. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT1 Conclusion

The inter-laboratory proficiency test 2014 was conducted without major constraints. 91% of parcels were delivered by the shipping companies within 8 days after submission. It was, however, unfortunate that one parcel was 27 days on the way and one parcel was 57 days on the way before delivered to the laboratory primarily due to border controls.

EHNV was included in the proficiency test for the first time in 2009. This year 40 participants were able to correctly identify the virus. Of the laboratories performing PCR based methods, 38 laboratories performed sequencing. Of these laboratories all correctly identified the content.

This year it has to be remarked that a problem with the batch of ampoules containing IPNV Ab has appeared, this has been taken into consideration in the process of giving score to participants. This year variation between virus titres obtained in the various laboratories was more pronounced than usually with up to 6 log differences between highest and lowest titres. It might reflect variation in the stability of the virus in the respective batches. Special precautions will therefore be taken in the following PT´s to ensure uniformity of the amount of viable viruses in the ampoules.

PT2 consisted of four coded ampoules (VI-IX). Two ampoules contained KHV, one ampoule contained ISAV and one Sterile Medium, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might had been possible to replicate them in cell cultures.

Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the providers of the proficiency test have included comments to the participants if relevant. An uncoded version of the report is sent to the European Commission.

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory. Collected accreditation data will not be presented in this report but will be presented at the 19th Annual Workshop of the NRLs for Fish Diseases May 2015 in Copenhagen. Participants were asked to reply latest November 21st 2014. Laboratory (EURL) for Fish Diseases.
The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 43.
The tests were sent from the EURL in the beginning of September 2013.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.

PT2 conclusion

Considering that this was the fifth time that the EURL provided a proficiency test on ISAV and KHV identification, we consider that most participants obtained very good results. All 39 laboratories testing for KHV identified the virus in ampoule VI and VIII! Out of 40 laboratories 39 laboratories identified Not KHV or ISAV in ampoule VII. With only one false positive this is much less than observed in the PTs from previous years. All 40 laboratories testing for ISAV identified the virus in ampoule IX. Thereby very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these past 5 years, especially in relation to the sensitivity, as this year the viral content in the ampoule was low. After the European Commission in autumn 2012 de-listed Epizootic Ulcerative Syndrome cause by *Aphanomyces invadans* it has been agreed not anymore to include this pathogen in the PT for fish diseases.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis as it is important that laboratories can

discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains.
Of the 24 laboratories sequencing the ISAV virus all found that the isolate was with deletion in segment 6 and thus not belong to HPR0. Some of the participant also noticed that this year the HPR13 isolate from the Faroes was used instead of the Gleasvaer isolate that we have included in all the former PT´s.
The results of the proficiency tests will be further discussed at this presentation.

EURL TRAINING COURSE FOR 201

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Abstract:

Also for 2015 the EURL for fish diseases will organize two training courses.

The courses available are:

- **Methods for implementation of surveillance procedures for listed fish diseases. The course will be held in week 41 from Monday the 5th to Friday the 9th of October**

- **Introduction to Histopathology in fish diseases. The course will be held in week 42 from 12th -15th October 2015**

The content of the training courses and the procedure to register will be described.

More information will be soon made available on the EURL website

http://www.eurl-fish.eu

EURL activities in 2014

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Abstract:

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN); and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia(VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus disease (KHVD).
The 18th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 3-4 June 2014 at the premises of the Veterinary Institute. A total of 53 participants from 32 countries attended over the two days period. There were five sessions with a total of 29 presentations, 2 of which were given by invited speakers, and a working group session. A report was submitted in August 2014.

Again this year an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU but there were also participants from countries outside EU. For the fifth year, the proficiency test consisted of two tests, PT1 and PT2. PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV + SVCV and IPNV. PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 41 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2015. Most laboratories performed well.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. Diagnostic manuals for VHS, IHN, ISA, KHV and EHN are available at the EURL web page.
In order to update the sampling and diagnostic procedures for KHV the EURL invited 3 experts for a 2 day scientific meeting and passed the outcome to the Commission in order to recommend and finally adopt a Commission Decision on KHV along with the other non-exotic aquatic animal diseases. The Commission Decision on sampling and diagnostic procedures for the listed non-exotic diseases are expected to be adopted by the Commission primo 2015 and will be finally in force as soon as accepted.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of these efforts was the implementation of a real time RT-PCR for detection of virusY, the putative causative agent of a new disease observed in Rainbow trout in Norway.

During 2014, resources were again used to collate data on surveillance, health categorisation, and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to produce anti-sera; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

In 2014 the fish diseases activities of DTU Veterinary were established in Copenhagen after the transfer from Aarhus in 2013. The number of colleagues within the group is now 5 academics and 4 technicians, but the EURL still collaborate with the fish diseases research group conducted by prof. Niels Lorenzen who, due to the transfer, jumped from DTU to Aarhus University.

The new placement also resulted in a close localization together with scientists conducting the function as NRL for mollusc diseases and who are internationally recognised researchers in fish bacteriology, as well as close distance to excellent scientists and research facilities in the Copenhagen area.

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EURL WORKPLAN FOR 2015

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1. Coordination and training			
1-1	Annual workshop	Organize and prepare for the 19th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2015	To be held 27-28 May 2015
1-2	Annual workshop report	Produce a technical and financial report from the Annual Workshop 2015.	To be finalized and submitted August 2015
1-3	Survey& diagnosis	Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2.	A questionnaire will be submitted in January 2015 and data collated for the Annual Workshop in May.
1-4	Training	Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered at the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.	Training courses are provided fall (temptatively November) 2015; two courses of 3-4-day each with expected 15 participants are foreseen.
1-5	Scientific working group	Organize specific scientific meeting collating international experts to assess and provide recommendations on emerging diseases problems management and control.	One meeting gathering 5 to 7 international experts will be held at our premises or on spot according to disease case. Scientific report and recommendations will be delivered afterwards to relevant stakeholders.

2. Proficiency test			
2-1	Proficiency tests	Prepare the Annual Inter-laboratory Proficiency Tests year 2015 for the NRLs. The tests will include VHSV, IHNV, EHNV, ISAV, and KHV and will also address other common viral pathogens in fish farming (IPNV, SVCV etc)	To be shipped fall 2015 (tentatively primo September)
2-2	PT report	Collate and analyze information gained from the Inter-laboratory Proficiency Tests	Report for the proficiency test 2014 will be submitted February 2015, while results of the 2015 test will be finally collated December 2015,

3. Reagents and products			
3-1	Supply of Reagents	Supply reference reagents to the NRLs in Member States.	Reagents as monoclonal antibodies, rabbit antisera, pathogen isolates or cell cultures are expected to be send to approx 15 laboratories in 2015
3-2	Production of reagents	Production of diagnostic reagents against selected pathogens when necessary	Diagnostic reagents (i.e. polyclonal antibodies raised in rabbit, monoclonal antibodies from stored hybridoma cells or In Situ Hybridization -ISH probes) will be produced according to demand
3-3	Pathogen library	Update and maintain a library of isolates of Infectious salmon anaemia virus (ISAV), Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Hematopoietic Necrosis virus (IHNV), Koi Herpes virus (KHV) and Enzootic Hematopoietic Necrosis virus (EHNV) and other relevant putative emerging fish pathogens.	The library will be updated with 10 to 20 pathogen isolates

4. Scientific advice and activities			
4-1	Webpage	Update the webpage for the EURL, www.eurl-fish.eu	Keep the webpage constantly updated, uploading relevant material (e.g. AW report, AW presentations, Training course report etc.,)
4-2	Diagnostic manuals	Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN and EUS on the EURL web page.	The diagnostic manuals are finally adopted by the Commission in 2014. A number of comments are expected from the Member States. The EURL will revise these and update the manuals accordingly
4-3	Fishreflabnet	Maintain and further develop the interactive network with the NRLs, Fishreflabnet, in order to promote a more proactive data sharing and communication with and between reference laboratories in member states.	The webpage and mailing list based platform for communication and data sharing will be continued with periodical updates sent to all members that subscribed.
4-4	Pathogen characterization	Identify and characterise selected isolates of listed viruses (pathogenicity testing in-vivo and in-vitro, serological and genetic characterisation).	The EURL receive every year strains and samples for corroboration of diagnostic results in EU Member states. Regularly these strains must be characterised properly as an emergency response to avoid unwanted spreading of new pathogens in EU
4-5	www.fishpathogens.eu	Update and expand www.fishpathogens.eu with more pathogens.	The database is a valuable tool for virus characterisation and molecular epidemiology. The more isolates included the stronger the tool. New databases on other listed and emerging pathogens are in the pipeline such as a database on SAV (pancreas disease and sleeping disease viruses). At least 50 new isolates are envisaged to be included and 1 new database opened.
4-6	Molecular epidemiology	Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens.	A study involving isolates from several Continental European countries is envisaged.
4-7	Real-time PCR	Assessment and standardisation of real-	Real-time PCR is a

		time PCR tests for the diagnosis, identification and typing of emerging and listed non-exotic and exotic fish diseases.	highly sensitive and specific tool for diagnosis and surveillance of a number of listed pathogens. Published and non-published methods will be assessed in our premises in order to offer validated protocols for the NRL´s
4-8	Emerging diseases	In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases	It is that more focus should be given to emerging diseases and rapid response. An assessment of risk for contracting and spreading emerging and re-emerging diseases in EU will be continued in 2015 (e.g. CEV – Koi sleepy disease; virusY in Rainbow trout, RLO-Rickettsia like organism in Sea bass)
4-9	Producing virtual teaching material (e-learning)	Preparing virtual guidelines for conducting proficiency tests (receiving and opening ampules inoculation etc.)	Set up tools for producing e-tutorials in-house. One tutorial on opening ampoules for PT produced.

5. Missions

5-1	Missions	Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial. As collaboration with NRLs in 3rd countries from where EU is importing large amount of fish products is increasing, missions to these, e.g. China and Korea is foreseen	1-2 missions will be conducted, the laboratories to visit will be appointed in order to strengthen collaboration in the NRL network. (e.g. Spain, Croatia, Iceland etc…)
5-2	International meetings	Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.	The EURL expect to participate in 2 to 3 international conferences.

DRAFT EURL WORKPLAN FOR 2016

Niels Jørgen Olesen, Susie Sommer Mikkelsen, Teena Klinge, Tine Iburg and Niccolò Vendramin

EURL FOR FISH DISEASES, 2016

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2016

1. Coordination and training

1-1 Organise and prepare for the 20th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2016. Cph primo June 2016?

1-2 Produce a report from the Annual Workshop 2016.

1-3 Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases. With more focus on emerging diseases.

1-4 Facilitate and provide training in laboratory diagnosis. The training courses in methods used for diagnosis of fish diseases is offered annually at the premises of the EURL-Fish. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants. The courses will be conducted autumn 2016

2. Proficiency test

2-1 Prepare the Annual Inter-laboratory Proficiency Tests year 2016 for the NRLs. The test will include testing for VHSV, IHNV, EHNV, ISAV, KHV and in addition upon request SVC and IPN. If accepted by the NRLs is will also include SAV.

2-2 Collate and analyse information gained from the Inter-laboratory Proficiency Tests

3. Reagents and products

3-1 Supply reference reagents to the NRLs in Member States.

3-2 Produce a panel of well characterized VHSV and IHNV isolates to be distributed to interested NRLs for e.g test validation and implementation.

3-3 Update and maintain a library of isolates of ISAV, VHSV, IHN, KHV, EHNV and pathogens causing disease that might be listed in future, e.g. SAV, nodaviruses.

3-4 Maintain a library of tissue material from fish infected with listed pathogens

4. Scientific advice and activities

4-1 Update and maintain the webpage for the EURL, www.eurl-fish.eu

4-2 Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, and EHN on the EURL web page.

4-3 Collect information on strain variation occurring within pathogens causing the listed diseases VHS, ISA, EHN and KHV disease and provide recommendations on how to discriminate between various strains.

4-4 Identify and characterise selected isolates of listed viruses (genetic characterisation).

4-5 Update and expand www.fishpathogens.eu with more pathogens.

4-6 Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.

4-7 In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases.

5. Missions

5-1 Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.

5-2 Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.

Apart from all the mandatory plans for next year suggestions for other topics to work on for the EURL would be most appreciated